AR201-13552A

HIGH PRODUCTION VOLUME (HPV) CHEMICAL CHALLENGE PROGRAM

TEST PLAN

for

ROSIN ESTERS

CAS No. 8050-26-8
CAS No. 8050-31-5
CAS No. 68153-38-8
CAS No. 68186-14-1
CAS No. 65997-13-9
CAS No. 64365-17-9
CAS No. 8050-15-5

Submitted to the US EPA

By

The Pine Chemicals Association, Inc.
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HPV Task Force
Consortium Registration # [redacted]

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Test Plan for Rosin Esters

Summary

The Pine Chemicals Association, Inc. (PCA) is sponsoring 36 HPV chemicals, including 19 members of the rosin family. Test plans for rosin and rosin salts (comprising six substances) and rosin adducts (comprising six substances) have previously been submitted.

This Test Plan addresses the following seven chemicals, known collectively as Rosin Esters:

CAS No. 8050-26-8, Rosin, pentaerythritol ester
CAS No. 8050-31-5, Rosin, glycerol ester
CAS No. 68153-38-8, Rosin, diethylene glycol ester
CAS No. 68186-14-1, Rosin, methyl ester
CAS No. 65997-13-9, Rosin, hydrogenated, glycerol ester
CAS No. 64365-17-9, Rosin, hydrogenated, pentaerythritol ester
CAS No. 8050-15-5, Rosin, partially hydrogenated, methyl ester

All of the members of this group of substances are closely related to rosin, a naturally occurring substance found in trees, predominantly pine trees. Rosin is composed primarily of resin acids, a class of tricyclic carboxylic acids, but also contains minor amounts of dimerized rosin, fatty acids and unsaponifiable matter. All the members of this group are esters of rosin that are made by reacting rosin with selected alcohols or polyols at elevated temperatures. As with other rosin-based products, these substances are complex mixtures and, therefore, are Class 2 substances.

The physical properties of rosin esters depend to a large extent on the hydroxy compound used to prepare the ester and can range from liquids to brittle solids. The largest end use for these rosin esters is as tackifiers in a wide variety of adhesive formulations. The specific rosin ester selected depends on the properties required in the final adhesive.

PCA has reviewed existing data on the compounds in this category. There are existing data on rosin, pentaerythritol ester; rosin, glycerol ester; rosin, hydrogenated, glycerol ester; and rosin, hydrogenated, pentaerythritol ester for many SIDS endpoints. The data demonstrate that these compounds are non-toxic in acute toxicity tests in multiple species. Existing data from repeat-dose studies, including long-term carcinogenicity studies, show low toxicity and no potential carcinogenic or reproductive effects.

Where applicable, PCA will conduct physical/chemical property and environmental fate testing on all of the substances in this category for which data are not already available. However, PCA has elected to treat this group of

chemicals as a category for purposes of the HPV program. Rosin, pentaerythritol ester (CAS# 8050-26-8) and rosin, partially hydrogenated, methyl ester (CAS# 8050-15-5) have been selected as the representative substances in this category for testing for the additional SIDS data. These two substances represent the extremes of the properties of the members of this category -- with the pentaerythritol ester having the highest molecular weight and the methyl ester, the lowest. Further, both of these substances are commercially important.

Two representatives of the category will be used for ecotoxicity and developmental toxicity testing. Most of the other required mammalian toxicity data (with the exception of acute oral toxicity) are available for one of the representatives of this category (rosin, pentaerythritol ester); therefore, additional mammalian toxicity testing will only be conducted on the other representative compound (rosin, partially hydrogenated, methyl ester).

A brief summary of the available data for the substances in this category, and the anticipated additional testing, is described below in Table 1.

Table 1
Matrix of Available Adequate Data and Proposed Testing
On Rosin Esters *

Chemical and CAS #	Required SIDS Endpoints										
	Partition Coef.	**Water Sol.**	**Biodeg.**	**Acute Fish**	**Acute Daph.**	**Acute Algae**	**Acute oral**	**Repeat Dose**	**In vitro genotox (bact.)**	**In vitro genotox (non-bact)**	**Repro/ Develop**
Rosin, penta-erythritol ester 8050-26-8	Test	Test	Test	Test	Test	Test	Test	Adeq.	Adeq.	Adeq.	Adeq. Repro; Test Develop.
Rosin, glycerol ester 8050-31-5	Test	Test	Test	C	C	C	Adeq.	Adeq.	Adeq.	Adeq.	Adeq. Repro; C Develop.
Rosin, diethylene glycol ester 68153-38-8	Test	Test	Test	C	C	C	C	C	C	C	C
Rosin, methyl ester 68186-14-1	Test	Test	Test	C	C	C	Adeq.	C	C	C	C
Rosin, hydrogenated glycerol ester 65997-13-9	Test	Test	Test	C	C	C	C	Adeq.	C	C	Adeq. Repro; C Develop
Rosin, hydrogenated penta-erythritol ester 64365-17-9	Test	Test	Test	C	C	C	C	Adeq.	C	C	Adeq. Repro; C Develop
Rosin, partially hydrogenated methyl ester 8050-15-5	Test	Test	Adeq.	Test	Test	Test	Adeq.	Test	Test	Test	Test Test

Adeq. Indicates adequate existing data
Test Indicates proposed testing
C Indicates category read-down from existing or proposed test data on rosin, pentaerythritol ester or rosin, partially hydrogenated, methyl ester

***** No testing will be conducted for melting point, boiling point, vapor pressure, hydrolysis, photodegradation and transport and distribution between environmental compartments as explained in the test plan.

Physical/Chemical Properties

Physical and chemical properties will be determined when appropriate. However, many of the physical and chemical properties are either inappropriate or cannot be measured for these compounds:

- Melting points will not be determined because these substances are complex mixtures and either will not give a sharp melting point when heated or will decompose before they melt.
- Boiling points cannot be determined because these substances are complex mixtures and will undergo oxidation or partial decomposition before they boil.
- Vapor pressure of these chemicals under ambient conditions is essentially zero and experimental measurement is not possible.
- Water solubility of all of the compounds in this category will be determined.
- Partition coefficients will be tested for all of the substances in this category. The partition coefficient testing likely will yield more than one value representing the various components, rather than a single value representing the mixture.

Environmental Fate

With respect to the SIDS environmental fate endpoints:

- Biodegradation data will be generated for six of the compounds in this category.
- Hydrolysis in water will not be determined for any of the compounds in this category because the members of this category are known to be highly resistant to hydrolysis.
- Photodegradation is not relevant, since the vapor pressure of these compounds is essentially zero and they could not enter the atmosphere.
- Transport and distribution between environmental compartments will not be determined due to the inability to provide usable inputs to the required model.

Ecotoxicity

- Existing ecotoxicity data are not reliable due to inconsistencies in, or artificial methods of, sample preparation. Acute toxicity to fish, daphnia and algae will be tested on the two representative members of this category under conditions that maximize solubility, but reduce exposure to insoluble fractions, which may cause nonspecific toxicological effects.

Mammalian Toxicity

- For the SIDS human health endpoints, there are adequate data on repeat dose toxicity, and reproductive effects for “rosin, pentaerythritol ester”; acute toxicity will be tested on this compound. For “rosin, partially hydrogenated, methyl ester,” there are adequate data on acute toxicity; this compound will be tested for repeat dose and reproductive toxicity.
- The availability of a two-year feeding study on “rosin, pentaerythritol ester” showing a lack of carcinogenicity obviates the need for *in vitro* genotoxicity testing. “Rosin, partially hydrogenated, methyl ester” will be tested for genotoxicity in a bacterial and mammalian system.
- Developmental toxicity studies on both category representatives will be undertaken to fulfill this SIDS endpoint.

The Pine Chemicals Association, Inc. HPV Task Force includes the following companies:

Akzo Nobel Resins
Akzo Nobel - Eka Chemicals Incorporated
Arizona Chemical Company
Asphalt Emulsion Manufacturers Association
Boise Cascade Corporation
Cognis Corporation
Crompton Corporation
Eastman Chemical Co. (including the former Hercules Inc. Resins Division)
Georgia-Pacific Resins Inc.
Hercules Incorporated
ICI Americas (including the former Uniqema)
Inland Paperboard & Packaging, Inc.
International Paper Co. (including the former Champion International Corporation)
Koch Materials Co.
McConnaughay Technologies, Inc.
Mead Corp.
Packaging Corporation of America
Plasmine Technology, Inc.
Raisio Chemicals
Rayonier
Riverwood International
Smurfit – Stone Container Corporation
Westvaco
Weyerhaeuser Co.

The Task Force will be filing multiple test plans covering various chemicals. Not all members of the Task Force produce the substances covered by this test plan.

I. Description of Rosin Esters

The Pine Chemicals Association, Inc. (PCA) is sponsoring seven HPV chemicals known collectively as Rosin Esters. This category of chemicals consists of the following:

CAS No. 8050-26-8, Rosin, pentaerythritol ester
CAS No. 8050-31-5, Rosin, glycerol ester
CAS No. 68153-38-8, Rosin, diethylene glycol ester
CAS No. 68186-14-1, Rosin, methyl ester
CAS No. 65997-13-9, Rosin, hydrogenated, glycerol ester
CAS No. 64365-17-9, Rosin, hydrogenated, pentaerythritol ester
CAS No. 8050-15-5, Rosin, partially hydrogenated, methyl ester

Rosin is a naturally occurring substance found in trees, predominantly pine trees. Rosin is composed primarily of rosin acids, a class of tricyclic carboxylic acids, but also contains minor amounts of dimerized rosin, fatty acids and unsaponifiable matter. Rosin and rosin salts are addressed in another test plan.

All the members of the category covered by this test plan are esters of rosin, made by reacting rosin with selected alcohols or polyols. The esterification reactions between rosin and various hydroxy compounds are shown schematically in Figures 1-4 below. These figures illustrate that the carboxylic acid group of the rosin reacts with the hydroxyl group of the alcohol or polyol, with the elimination of water.

In order for esterification to take place, the chemical reactions for producing the various rosin esters are carried out at elevated temperatures to remove the water of reaction. With esterification reactions involving polyols, temperatures in excess of 250 ^{o}C are generally required in order to force the reaction towards completion. Because the rosin molecule is very large compared to the small polyol molecules and because the acid group of rosin is tertiary, a great amount of energy is required to overcome the steric effects. In actual practice, complete esterification is never achieved and all rosin esters contain small amounts (ca 5%) of unreacted rosin (Zinkel and Russell 1989).

As with other rosin-based products, these substances are complex mixtures and, therefore, are Class 2 substances.

A. Composition

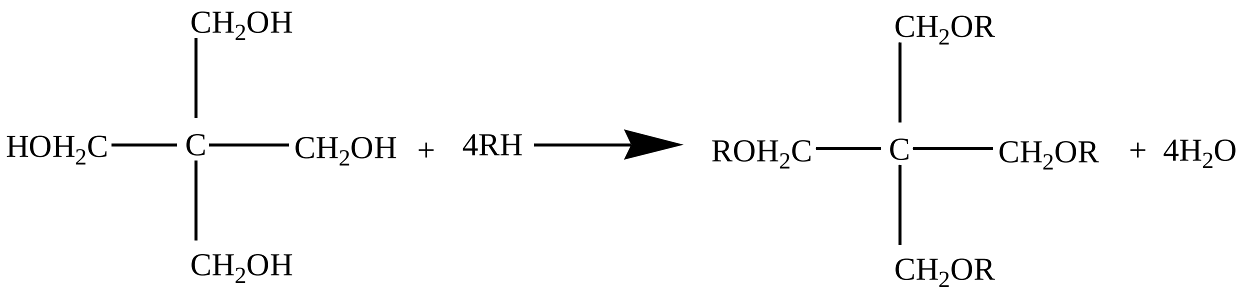
The physical properties of rosin esters depend to a large extent on the hydroxy compound used to prepare the ester and can range from liquids to brittle solids. The largest end use for these rosin esters is as tackifiers in a wide variety of adhesive formulations. Various rosin esters are used in solvent-based, water-based and hot-melt adhesives, with the specific ester selected dependent on the properties required in the final adhesive.

As previously noted, rosin esters are synthesized from rosin that is derived primarily from pine trees. The composition of rosin was described in the PCA's test plan for Rosin and its Salts, and the reader is referred to that plan for detailed information. The general characteristics and composition of the rosin esters in this category are addressed below.

1. Rosin, pentaerythritol ester (CAS# 8050-26-8) and Rosin, hydrogenated, pentaerythritol ester (CAS# 64365-17-9)

These substances are made by reacting rosin or hydrogenated rosin with pentaerythritol at a temperature of about 270^{0}C. The reaction is shown schematically in Figure 1. Pentaerythritol, with four hydroxyl groups, is capable of forming a tetraester. Because of steric effects, the reaction is not completely achieved even at the high temperatures used. Therefore, the commercial products are primarily a combination of tetraesters and triesters, with some di and mono ester, as well as a small amount of unreacted rosin. The only difference between the esters of rosin and hydrogenated rosin is that, in hydrogenated rosin ester, the double bonds in the rosin are removed prior to esterification with the aim of giving the final ester greater oxidative stability.

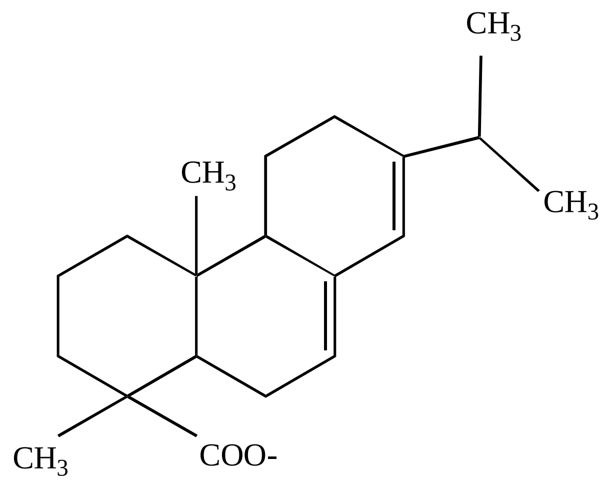
The substances are brittle glass-like solids ranging in color from very pale yellow to pale brown. They do not have a true melting point, but they have a softening point of about 100^{0}C.



Pentaerythritol

Pentaerythritol ester of rosin

where R is



or other similar rosin acid

Figure 1. Formation of the pentaerythritol ester of rosin

2. Rosin, glycerol ester (CAS# 8050-31-5) and Rosin, hydrogenated, glycerol ester (CAS# 65997-13-9)

These substances are made by reacting rosin or hydrogenated rosin with glycerol at a temperature of about 250^{0}C. The reaction is shown schematically in Figure 2. Glycerol, with three hydroxyl groups, is capable of forming a triester. Because of the steric effects of the reaction, the reaction cannot be completely achieved even at the high temperatures used, and the commercial products are primarily a combination of triesters and diesters, with small amounts of monoesters and unreacted rosin. Again, the only difference between the glycerol esters of rosin and hydrogenated rosin is that, in the latter, the double bonds in the rosin are removed prior to esterification with the aim of giving the final ester greater oxidative stability.

Like the pentaerythritol esters, these esters are also brittle glass-like solids ranging in color from very pale yellow to pale brown. They do not have a true melting point but they have a softening point of about 85^{0}C.

$$\begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OH \end{array} \quad + \quad 3RH \longrightarrow \begin{array}{l} CH_2OR \\ | \\ CHOR \\ | \\ CH_2OR \end{array} \quad + \quad 3H_2O$$

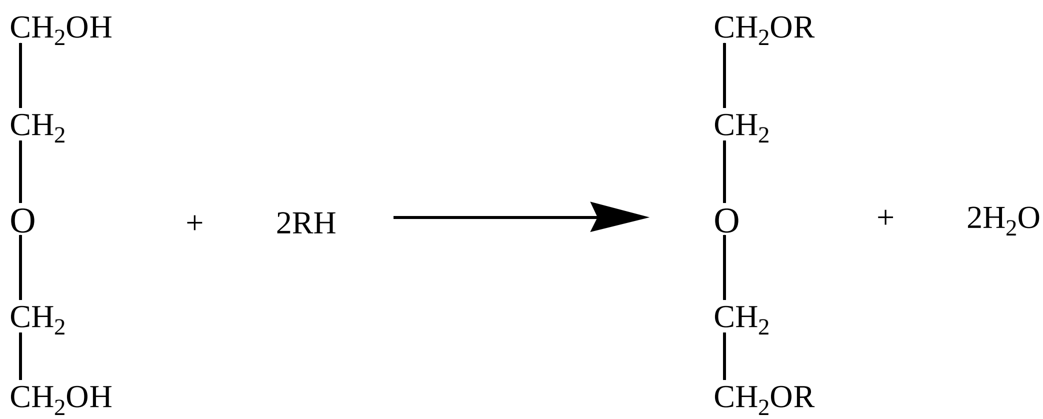
Glycerol — Glycerol ester of rosin

where R is as defined in Figure 1

Figure 2. Formation of the glycerol ester of rosin

3. Rosin, diethylene glycol ester (CAS# 68153-38-8)

This substance is made by reacting rosin or hydrogenated rosin with diethylene glycol at a temperature of about 250^{O}C. The reaction is shown schematically in Figure 3. Diethylene glycol, with two hydroxyl groups, is capable of forming a diester. As a consequence of steric effects, the commercial products contain both di and mono esters, as well as a small amount of unreacted rosin. The substance is a viscous liquid at room temperature and is pale yellow in color.



diethylene glycol

diethylene glycol ester of rosin

where R is as defined in Figure 1.

Figure 3. Formation of the diethylene glycol ester of rosin

4. Rosin, methyl ester (CAS# 68186-14-1) and Rosin, partially hydrogenated, methyl ester (CAS# 8050-15-5)

These substances are made by reacting rosin or hydrogenated rosin with methanol at an elevated temperature. These reactions are carried out at a lower temperature than the glycerol or pentaerythritol esterifications because of the low boiling point of methanol. In this case, the reversible reaction is forced toward the ester by using excess alcohol as well as elevated temperature. The reaction is shown schematically in Figure 4. Because methanol is monohydric, only one ester is formed. Again, the only difference between the methyl esters of rosin and hydrogenated rosin is that, in the latter, the double bonds in the rosin are removed prior to esterification with the aim of giving the final ester greater oxidative stability. The methyl esters are free-flowing liquids with colors ranging from almost water white to pale yellow.

$$\underset{\text{Methanol}}{CH_3OH} \quad + \quad RH \longrightarrow \underset{\text{Methyl ester of rosin}}{CH_3OR} \quad + \quad H_2O$$

where R is as defined in Figure 1.

Figure 4. Formation of the methyl ester of rosin.

B. Commercial Uses of Rosin Esters

Esters of rosin are found in several different end use markets, especially hot melt and pressure sensitive adhesives, and chewing gum. Hot melt adhesives are a major use area for rosin esters. Applications include all types of packaging, book-binding, and disposable diaper construction. Tackifiers used for hot melt adhesives are primarily pentaerythritol esters. These are preferred over glycerol esters in hot melt applications primarily due to oxidative resistance combined with higher softening points. Aqueous dispersions of rosin esters are used in the rapidly growing pressure sensitive adhesives market. Simple glycerol esters of rosin are used in chewing gum as a tackifier. These substances are approved for use by FDA as direct food additives in chewing gum under 21 CFR § 172.615 (a).

C. Complexity of Analytical Methodology

All the substances in this category are Class 2 substances. This, combined with fact that they are essentially insoluble in water and, with two exceptions, decompose rather than vaporize on heating creates a variety of analytical challenges. Gas chromatography is applicable to the analysis of the two methyl esters but not the other esters. The most feasible approach for the analysis of the non-methyl esters is thought to be size exclusion gel permeation chromatography. Although this technique separates components based on size rather than chemical composition, preliminary studies indicate that it will be generally applicable to the non methyl esters. The solubility of rosin esters is so low (ca 10 ppm) that the reliability of this analytical method at such low concentrations has to be established. However, based on the method validation work to date, it appears that the analytical procedures available will be adequate for the proposed testing.

II. Rationale for Selection of Representative Compounds for Testing

Rosin, pentaerythritol ester (CAS# 8050-26-8) and rosin, partially hydrogenated, methyl ester (CAS# 8050-15-5) have been selected as the representative substances in this category for testing for the applicable SIDS ecotoxicity and developmental toxicity tests, as shown in Table 2 (identical to Table 1). As further indicated in Table 2, pertinent physical/chemical properties and environmental fate endpoints will be determined for all members of this category where data are not already available.

These two substances represent the extremes of the properties of the members of this group. Pentaerythritol ester has the highest molecular weight and the methyl ester, the lowest. This molecular weight range manifests itself with the pentaerythritol ester having the highest softening point and the methyl ester the lowest. Consequently, the selection of these two substances as representatives of this category is consistent with the EPA guidelines since their molecular weights bracket the category. Further, both of these substances are commercially important, with the pentaerythritol ester being one of the highest volume rosin derivatives produced in the United States.

Another criterion listed by EPA for grouping chemicals into a category is the use of the "family approach" of examining related chemicals. Since all of the chemicals in this category are esters of rosin, they are in the same family of compounds. In summary, this group of chemicals fits the requirements of the EPA's HPV Challenge program for a chemical category, and rosin, pentaerythritol ester and rosin, partially hydrogenated, methyl ester are the most appropriate representative test materials from this category.

III. Review of Existing Data and Development of Test Plan

PCA has undertaken a comprehensive evaluation of all relevant data on the SIDS endpoints of concern for the chemicals in this category. Considerable data are available that satisfy many of the SIDS endpoints for this category. The availability of the data on the specific SIDS endpoints is summarized in Table 2 (identical to Table 1). Table 2 also shows data gaps that will be filled by additional testing, and areas where data from rosin, pentaerythritol ester and rosin, partially hydrogenated, methyl ester will be generalized to other category members. In addition, as can be seen in Table 2, there are also data on other members of this category.

Table 2
Matrix of Available Adequate Data and Proposed Testing
On Rosin Esters*

Chemical and CAS #	Required SIDS Endpoints										
	Partition Coef.	**Water Sol.**	**Biodeg.**	**Acute Fish**	**Acute Daph.**	**Acute Algae**	**Acute oral**	**Repeat Dose**	**In vitro genotox (bact.)**	**In vitro genotox (non-bact)**	**Repro/ Develop**
Rosin, penta-erythritol ester 8050-26-8	Test	Test	Test	Test	Test	Test	Test	Adeq.	Adeq.	Adeq.	Adeq. Repro/ Test Develop.
Rosin, glycerol ester 8050-31-5	Test	Test	Test	C	C	C	Adeq.	Adeq.	Adeq.	Adeq.	Adeq. Repro/ C Develop
Rosin, diethylene glycol ester 68153-38-8	Test	Test	Test	C	C	C	C	C	C	C	C
Rosin, methyl ester 68186-14-1	Test	Test	Test	C	C	C	Adeq.	C	C	C	C
Rosin, hydrogenated glycerol ester 65997-13-9	Test	Test	Test	C	C	C	C	Adeq.	C	C	Adeq. Repro/ C Develop
Rosin, hydrogenated penta-erythritol ester 64365-17-9	Test	Test	Test	C	C	C	C	Adeq.	C	C	Adeq. Repro/ C Develop
Rosin, partially hydrogenated methyl ester 8050-15-5	Test	Test	Adeq.	Test	Test	Test	Adeq.	Test	Test	Test	Test Repro/ Test Develop

Adeq. Indicates adequate existing data
Test Indicates proposed testing
C Indicates category read-down from existing or proposed test data on rosin, pentaerythritol ester or rosin, partially hydrogenated, methyl ester.
***** No testing will be conducted for melting point, boiling point, vapor pressure, hydrolysis, photodegradation and transport and distribution between environmental compartments as explained in the test plan.

A. Evaluation of Existing Physicochemical Data and Proposed Testing

The basic physicochemical data required in the SIDS battery includes melting point, boiling point, vapor pressure, partition coefficient (K_{ow}), and water solubility.

Class 2 substances are composed of a complex mixture of substances and are often difficult to characterize. The rosin esters are not only Class 2 substances, but also are derived from natural sources. Their composition is variable and cannot be represented by a single chemical structural diagram. Due to this "complex mixture" characteristic of rosin esters, some physical property measurements, such as partition coefficient, do not give single definitive results because the methodology used to determine these properties will actually fractionate or partition the substance into various components. Since the methodology will alter the actual sample composition, the results are likely to be erroneous, difficult to interpret, or meaningless.

1. **Melting Point**

Due to their complex nature, the members of this category soften over a range of temperature and do not have a well defined melting point. Consequently, the melting point of these substances will not be measured.

2. **Boiling Point**

All of the members of this category are produced at high temperatures, and are non-volatile solids or liquids at ambient temperatures. A boiling point at ambient pressure has no meaning because these materials will oxidize or decompose before they boil. Accordingly, measurement of this property is inappropriate for all the substances in this category.

3. **Vapor Pressure**

Vapor pressures for rosin esters at ambient temperatures are effectively zero, and their experimental measurement is inappropriate.

4. **Water Solubility**

The water solubility of all of the compounds in this category will be determined using OECD (105).

5. **Partition Coefficient**

The partition coefficient (i.e., K_{ow}) for all of the compounds in this category will be determined using OECD method 107. Adequate data exist for rosin, pentaerythritol ester and rosin, glycerol ester although both will be retested with the other compounds in this category. Existing data on rosin, pentaerythritol ester, demonstrate that two K_{ow} values, rather than a single value, are generated when this endpoint is determined. This outcome reflects the complex nature of Class 2 mixtures.

Summary of Physicochemical Properties Testing: The water solubility (OECD 105) and partition coefficients (OECD 107) of all of the substances in this category will be determined. Tests for melting point, boiling point, and vapor pressure are inapplicable to these substances.

B. Evaluation of Existing Environmental Fate Data and Proposed Testing

The fate or behavior of a chemical in the environment is determined by the reaction rates for the most important transformation (degradation) processes. The basic environmental fate data covered by the HPV Program include biodegradation, stability in water (hydrolysis as a function of pH), photodegradation and transport and distribution between environmental compartments.

1. Biodegradation

Biodegradability provides a measure for the potential of compounds to be degraded by microorganisms. Depending on the nature of the test material, several standard test methods are available to assess potential biodegradability.

One of the chemicals in this category (rosin, partially hydrogenated, methyl ester) has existing data on the biodegradation endpoint. Biodegradation for six compounds will be determined using OECD protocol 301B.

2. Hydrolysis

Hydrolysis as a function of pH is used to assess the stability of a substance in water. Hydrolysis is a reaction in which a water molecule (or hydroxide ion) substitutes for another atom or group of atoms present in an organic molecule. Experience has shown that rosin ester molecules are very resistant to hydrolysis. The rosin esters will hydrolyze only under extreme laboratory conditions (i.e., strong alkali and elevated temperatures) which are not normally found in the environment nor are such conditions part of the OECD test protocol.

In addition, low water solubility often limits the ability to determine hydrolysis as a function of pH. All of the rosin esters have very low solubility in water. Therefore, these materials are expected to be stable in water and it would be unnecessary to attempt to measure the products of hydrolysis.

3. Photodegradation

Due to their lack of any vapor pressure under ambient conditions, there is essentially no opportunity for any of these chemicals to enter the atmosphere. Thus, photodegradation is irrelevant. In addition, based on the constituents in these complex mixtures, there is no reason to suspect that they would be subject to breakdown by a photodegradative mechanism. Consequently, this endpoint will not be determined for any of the substances in this category.

4. Transport and Distribution between Environmental Compartments

The transport and distribution between environmental compartments is intended to determine the ability of a chemical to move or partition in the environment. The determination of this property requires the use of various models (e.g., level III model from the Canadian Environment Modeling Centre at Trent University). For Class 2 substances such as the rosin esters, the required inputs to the model are either not available or not feasible to determine including molecular mass, reaction half-life estimates for air, water, soil, sediment, aerosols, suspended sediment, and aquatic biota. In addition, while the partition coefficient is also required and can be determined, the multiple K_{ow} values typically derived for these substances are a consequence of sample fractionation and reflect various components in the mixture and are not representative of the mixture itself. Consequently, due to the inability to provide usable inputs to the required model, no determination of transportation and distribution between environmental compartments will be undertaken for rosin esters.

Summary of Environmental Fate Testing: Biodegradation data will be generated (using OECD 301B) for six of the compounds in this category. Photodegradation, hydrolysis and transport and distribution between environmental compartments are not applicable to these chemicals.

C. Evaluation of Existing Ecotoxicity Data and Proposed Testing

The basic ecotoxicity data that are part of the HPV Program include acute toxicity to fish, daphnia and algae. While there are some existing data on these endpoints for substances in this category, these data are conflicting and it is impossible to determine which, if any, of these findings is representative of true ecotoxicity. The inconsistencies in how water samples were prepared for testing these endpoints render these data inadequate. Consequently, rosin, pentaerythritol ester and rosin, partially hydrogenated, methyl ester will be tested for acute toxicity to fish, daphnia and algae under conditions that maximize the solubility under the specific test exposure conditions, but reduce exposure to insoluble fractions, which may cause nonspecific toxicological effects. In addition, the effect of both filtering, to further minimize nonspecific physical effects, and of reducing the pH to the lower end of the acceptable range for test organism survival, will also be investigated for changes in toxicological effects. The results of preliminary tests will be used to select the most appropriate test conditions for the definitive test for each species.

Summary of Ecotoxicity Testing: The acute toxicity of rosin, pentaerythritol ester and rosin, partially hydrogenated, methyl ester to fish (OECD 203), daphnia (OECD 202) and algae (OECD 201) will be tested under

conditions that maximize solubility, but reduce exposure to insoluble fractions, which may cause nonspecific toxicological effects.

D. Evaluation of Existing Human Health Effects Data and Proposed Testing

1. Acute Oral Toxicity

Acute oral toxicity studies investigate the effect(s) of a single exposure to a relatively high dose of a substance. This test is conducted by administering the test material to animals (typically rats or mice) in a single gavage dose. Harmonized EPA testing guidelines (August 1998) set the limit dose for acute oral toxicity studies at 2000 mg/kg body weight. If less than 50 percent mortality is observed at the limit dose, no further testing is needed. A test substance that shows no effects at the limit dose is considered essentially nontoxic. If compound-related mortality is observed, then further testing may be necessary.

Summary of Available Acute Oral Toxicity Data

One of the representative compounds, rosin, partially hydrogenated, methyl ester, as well as rosin, methyl ester are non-toxic following acute oral exposure with LD_{50} values > 2,000 mg/kg in rats, guinea pigs and rabbits. The other representative compound, rosin, pentaerythritol ester, will be tested for acute toxicity.

Summary of Acute Oral Toxicity Testing: One of the representative compounds in this category (rosin, partially hydrogenated, methyl ester) has been tested for acute oral toxicity and found to be non-toxic (i.e., LD_{50} > 2000 mg/kg). The other representative compound in this category (rosin, pentaerythritol ester) will be tested for this endpoint using OECD method 425.

2. Repeat Dose Toxicity

Subchronic repeat dose toxicity studies are designed to evaluate the effect of repeated exposure to a chemical over a significant period of the life span of an animal. Typically, the exposure regimen in a subchronic study involves daily exposure (at least 5 consecutive days per week) for a period of not less than 28 days or up to 90 days (i.e., 4 to 13 weeks). The HPV program calls for a repeat dose test of at least 28 days. The dose levels evaluated are lower than the relatively high doses used in acute toxicity (i.e., LD_{50}) studies. In general, repeat dose studies are designed to assess systemic toxicity, but the study protocol can be modified to incorporate evaluation of potential adverse reproductive and/or developmental effects.

Summary of Available Repeat Dose Toxicity Data

Existing data demonstrate low toxicity for rosin, pentaerythritol ester; rosin, glycerol ester; rosin, hydrogenated, glycerol ester; and rosin, hydrogenated, pentaerythritol ester in repeat dose tests.

Rosin, pentaerythritol ester was tested in a 90-day subchronic toxicity study in rats. The test material was administered to male and female Sprague-Dawley rats at dietary concentrations of 0, 0.01, 0.05, 0.2, 1, or 5% for 90 days. The approximate doses were 0, 10, 50, 200, 1,000, or 5,000 mg/kg/day. Parameters evaluated included mortality, clinical signs, body weight, body weight gain, food utilization, food consumption, hematology, urinalysis, gross pathology, organ weights, and microscopic pathology.

Treatment did not affect body weight, body weight gain, clinical signs, hematology, urinalysis, gross or microscopic pathology. Food consumption was decreased at 5%, but food utilization was unaffected suggesting that the decrease in consumption was related to palatability. Absolute and relative liver weights were significantly increased in the high-dose males and females; however, no changes were observed at histopathology. Based on these data, the no observed effect level (NOEL) was 1% (approximately 1,000 mg/kg/day) Other 90-day subchronic studies confirm the low toxicity of rosin, pentaerythritol ester (see robust summaries).

In addition, other chemicals in this category (rosin, glycerol ester; rosin hydrogenated, glycerol ester; and rosin, hydrogenated, pentaerythritol ester) have also been confirmed to have low toxicity in 90-day subchronic studies. In these studies, the only effects noted were either death due to palatability resulting in non-consumption of food or depression of body weight gain at the highest doses tested. The NOELs in these studies ranged from approximately 1000 to 2,500 mg/kg/day.

Summary of Repeat Dose Toxicity Testing: Rosin, pentaerythritol ester has been tested for repeat dose toxicity in several 90-day studies. In these studies, the NOELs were approximately 1000 mg/kg/day, indicating that this compound has low toxicity. Rosin, partially hydrogenated, methyl ester will be tested for repeat dose toxicity (in conjunction with reproductive and developmental toxicity) using OECD method 422 which reduces the number of animals used.

3. Genotoxicity – In vitro

Genetic testing is conducted to determine the effects of substances on genetic material (i.e., DNA and chromosomes). The gene, which is composed of DNA, is the simplest functional genetic unit. Mutations of genes can occur spontaneously or as a consequence of exposure to chemicals or radiation. Genetic mutations are commonly measured in bacterial and mammalian cells, and the HPV program calls for completing both types of tests.

Summary of Available Genotoxicity Data

Rosin, pentaerythritol ester has been tested for potential carcinogenicity in a two-year bioassay conducted in rats. This study did not demonstrate any evidence of carcinogenicity. The primary effect was depressed weight gain at the highest dose, confirming that a maximally tolerated dose was achieved.

Since the purpose of *in vitro* bacterial and mammalian mutagenicity tests is to determine if a chemical might have the potential to be a direct-acting DNA reactive carcinogen, the negative carcinogenicity study eliminates the need to test rosin, pentaerythritol ester for potential genotoxicity.

In addition, rosin, glycerol ester has been tested for genotoxicity in several test systems including the Ames *Salmonella* assay, chromosomal aberrations in Chinese hamster ovary (CHO) cells and a rat primary hepatocyte assay to measure unscheduled DNA synthesis. None of these test systems showed any indication of genotoxicity. Rosin, partially hydrogenated methyl ester has not been tested for genotoxicity.

Summary of Genotoxicity Testing: Because rosin, pentaerythritol ester was not carcinogenic in a two-year cancer bioassay, no genotoxicity testing is necessary for this compound. Rosin, partially hydrogenated, methyl ester will be tested for genotoxicity in bacteria (OECD 471) and mammalian cells (OECD 476).

4. Reproductive and Developmental Toxicity

Reproductive toxicity includes any adverse effect on fertility and reproduction, including effects on gonadal function, mating behavior, conception, and parturition. Developmental toxicity is any adverse effect induced during the period of fetal development, including structural abnormalities, altered growth and post-partum development of the offspring.

The “toxicity to reproduction” aspect of the HPV Challenge Program can be met by conducting a reproductive/developmental toxicity screening test or adding a reproductive/developmental toxicity screening test to the repeat dose study (OECD 421 or OECD 422, respectively).

Summary of Reproductive/Developmental Toxicity Data

As noted in the SIDS guidelines for the reproduction toxicity endpoint, *"when a 90-day repeated dose study is available and demonstrates no effects on the reproductive organs, in particular the testes, then a developmental study can be considered as an adequate test to complete information on reproduction/developmental effect."* The following rosin esters have been tested in 90-day repeat dose studies: rosin, pentaerythritol ester; rosin, glycerol ester;

rosin, hydrogenated, glycerol ester; and rosin, hydrogenated, pentaerythritol ester. In addition, rosin pentaerythritol ester has also been tested in a two-year bioassay. All of the 90-day studies and the two-year study included histopathology of reproductive organs (i.e., testes, ovaries, uterus).

One 90-day study on rosin, pentaerythritol ester reported testicular toxicity at 5,000 mg/kg/day; no adverse reproductive effects were observed at lower doses (i.e., 1,000 mg/kg/day and below). This result could not be replicated in a second 90-day study on rosin, pentaerythritol ester which revealed no testicular effects at doses up to and including 5,000 mg/kg/day. In addition, the two-year study showed no evidence of reproductive toxicity. The weight-of-evidence, i.e., (1) the lack of dose-response, (2) the lack of reproductive effects in a second study using the same compound, doses and design, and (3) the lack of reproductive effects in studies conducted on other rosin esters suggests that the testicular toxicity observed in the rosin, pentaerythritol ester study was an isolated finding and is not representative of the class of rosin esters.

Based on these data, it is concluded that the database of studies for the rosin esters satisfies the SIDS reproductive toxicity endpoint for one of the representative compounds. A developmental toxicity study using OECD Method 421 will be conducted on rosin, pentaerythritol ester to complete the information on reproductive/developmental toxicity.

Because there are no reproductive/developmental data for the other representative compound, rosin, partially hydrogenated, methyl ester, it will be tested for reproductive/developmental toxicity (in conjunction with repeat dose toxicity) using OECD method 422.

Summary of Reproductive/Developmental Testing: One repeat dose study on rosin, pentaerythritol ester showed testicular effects at the highest dose tested (5,000 mg/kg/day). This result could not be replicated in another repeat dose study at the same dose level nor was it observed in a two-year study. Since neither of these studies evaluated potential developmental toxicity, rosin, pentaerythritol ester will be tested for this endpoint with OECD method 421. Rosin, partially hydrogenated, methyl ester will be tested for reproductive/developmental toxicity using OECD method 422. Combining the testing in a single protocol will require the use of fewer animals.

References

Zinkel, D.F. and Russell, J., Eds. 1989. Naval Stores. Production, Chemistry, Utilization. Pulp Chemicals Association, New York.

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